

# Inoculum Reduction Measures to Manage Armillaria Root Disease in a Severely Infected Stand of Ponderosa Pine in South-Central Washington: 35-Year Results

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## ABSTRACT

A stand of ponderosa pine (*Pinus ponderosa*) severely affected by Armillaria root disease was treated with five different levels of sanitation by root removal to reduce root disease losses in the regenerating stand. Treatments included the following: (1) all trees pushed over by machine, maximum removal of roots by machine ripping, and visible remaining roots removed by hand; (2) all trees pushed over by machine and maximum removal of roots by machine ripping; (3) all trees pushed over by machine with no further removal of roots; (4) smaller trees pushed over by machine but large stumps left, otherwise maximum removal of roots by machine ripping; and (5) all trees felled and removed by skidding, area cleared of slash, sod scalped, and no removal of roots. After 35 years, we found that the more intense and thorough root-removal treatments were generally more effective in reducing the occurrence of Armillaria root disease. However, even the most intensive treatment (treatment 1), which experienced significantly less disease than most other treatments, had 23% of the area expressing mortality. The only operationally feasible treatment (treatment 3) also reduced levels of mortality, but not significantly (40% mortality versus 52% in the control, treatment 5). Although these results support the concept that inoculum removal can reduce root disease levels, the treatment necessary to provide a meaningful reduction in disease loss does not seem to warrant its cost.

**Keywords:** root disease control, inoculums reduction, disease spread, sanitation

In 1971 a long-term experiment was initiated to evaluate various levels and types of inoculum reduction, through stump and root removal, to control root disease in a ponderosa pine (*Pinus ponderosa*) forest heavily affected by *Armillaria ostoyae* (Shaw and Roth 1976, Shaw et al. 1976, Shaw 1980, Reaves et al. 1993) [1]. We believe this is the longest running experiment of its type in the United States; there is a similar experiment in British Columbia that is a few years older (Morrison et al. 1988, Morrison 1998).

As Filip et al. (2010) recently discussed, the common practice of selective harvesting in ponderosa pine stands, especially removal of large trees, may exacerbate Armillaria root disease owing to an increase of fungal inoculum in infected stumps of harvested trees (Roth et al. 1977, 1980, 2000, Shaw 1980). Inoculum longevity is proportional to inoculum (stump) size; thus, inoculum associated with larger infected stumps remains viable longer and therefore has a higher likelihood of promoting fungal spread to healthy trees. Large infected stumps thus have advantages of space and time to spread disease: Pathogenic fungi in larger root systems (space) can infect healthy trees over a longer period (time). Large stumps of ponderosa pine can contain viable mycelia of *Armillaria* for more than 35 years (Roth et al. 1980), and roots of recently killed large trees can extend >30 m from the stump in relatively open stands (Shaw and Roth 1976, Shaw et al. 1976).

The study site in south-central Washington contains a particularly large and virulent genet of *A. ostoyae* (Shaw and Roth 1976, Shaw 1977, Shaw et al. 1992) that severely affects a normally tolerant host species, ponderosa pine (Goheen and Willhite 2006, Filip et al. 2010). At least two other genets of *A. ostoyae* have been identified in the area (Anderson et al. 1979). The site was part of the “Humongous Fungus” debate of the early 1990s (Shaw et al. 1992). This study continues to test the hypothesis that disruption and removal of *Armillaria*-infected stumps and roots will decrease mortality and increase growth of ponderosa pine in the new stand.

The 20-year results from this experiment have been previously reported (Roth et al. 2000). Results after 20 years showed a general reduction in mortality caused by root disease with improved sanitation (i.e., levels of root material that serve as disease inoculum removed). The one treatment among those evaluated that was considered to be the most practical for general forestry application was “push-over” logging. In this treatment, all standing trees were pushed over prior to bucking, which dislodged from the soil major portions of the root system containing fungal inoculum. These dislodged root systems were removed from the site without any further attempt to remove additional roots (see Figure 4 in Roth et al. 2000).

This push-over treatment gave less than desired levels of control 20 years after application in that, on average, nearly one-third of the

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This article uses metric units; the applicable conversion factors are: centimeters (cm): 1 cm = 0.39 in.; meters (m): 1 m = 3.3 ft; kilometers (km): 1 km = 0.6 mi.

treated area expressed some tree mortality from *Armillaria* root disease (Roth et al. 2000). In contrast, the most thorough, but highly impractical, treatment, in which dislodged root residues were hand-picked from the site following stump removal and root raking by bulldozer, averaged less than half of this disease level. Disease occurred in more than 40% of the stand treated by clear felling with no root removal (Roth et al. 2000).

In 1992–1993, the study site was uniformly thinned so that each experimental unit (each 3.3-m<sup>2</sup> cell) within the treatment blocks that was still stocked was left with one ponderosa pine tree, yielding an approximate 3.6 × 3.6-m spacing. Whereas this action reduced the bioassay opportunity for root disease detection, it left a more realistic stand density for ponderosa pine at this age and site index (32 m at 100 years). Also, thinning of small-diameter pine on *Armillaria*-infested sites has been shown to significantly reduce leave-tree mortality after 30 years in central Oregon (Filip et al. 2009). In this article, results of the root removal treatments to control root disease 35 years after application and 15 years after the general thinning are reported and discussed in context with other root disease control efforts.

## Methods

The rather complex experimental design and root-removal treatments used in this study are thoroughly described and diagrammed in Roth et al. (2000). To briefly summarize, there were six root-removal treatments, each replicated three times in a block design across the severely diseased study site (see Figure 3 in Roth et al. 2000). The study area is located 10 km west of Glenwood, Washington (see Figure 2 in Roth et al. 2000) at about 900 m elevation in a ponderosa pine–Douglas-fir (*Pseudotsuga menziesii*)–*Carex* plant association (Franklin and Dyrness 1988) at T6N, R11E, Sec. 10, or 46.0224128 N, 121.4171362 W.

The root-removal treatments applied in summer 1971 were as follows: (1) all trees pushed over by machine, maximum removal of roots by machine ripping, and visible remaining roots removed by hand; (2) all trees pushed over by machine and maximum removal of roots by machine ripping; (3) all trees pushed over by machine with no further removal of roots; (4) smaller trees pushed over by machine but large stumps left, otherwise maximum removal of roots by machine ripping; (5) all trees felled and removed by skidding, area cleared of slash, sod scalped, and no removal of roots; and (6) only merchantable trees felled and removed with no further treatment. Trees in treatment 6 provided abundant seed to regenerate the site, but because the treatment differed so markedly in stocking and ground cover from the other treatments, it was not used in the earlier (Roth et al. 2000) or current analysis.

Across these root-removal treatments, some portions of the study were planted (from different seed sources) and some were thinned in 1977 and 1981, as described in Roth et al. (2000). This yielded a thinned, unthinned, and planted matrix within the root-removal treatments that was maintained in portions of the current analysis, even though all treatments, including the previously unthinned portion of the matrix, were thinned to an approximate 3.6 × 3.6-m spacing (one tree per stocked cell) in 1992–1993. As also described in Roth et al. (2000), an attempted seeding experiment failed and was removed from further analysis.

Each treatment/replication had 112 cells (each 3.3-m<sup>2</sup>) in which effectiveness of the sanitation treatments was evaluated by annually counting mortality. Periodically since the 1980s, the designated

leave tree in each cell, and the only tree remaining after 1992–1993, was measured for height and diameter.

Death of any tree in a cell from *Armillaria* root disease designated that cell as infested, with the actual mortality levels likely reflecting the minimum component of cells with *Armillaria* present. After 1992–1993, any mortality would leave a cell unstocked, whereas prior to 1992–1993, mortality could have left a cell stocked or unstocked. Data on effectiveness of root-removal treatments were analyzed by presence of *Armillaria*-caused mortality in any cell (see Table 2 in Roth et al. 2000) and by current (2007) stocking condition. The current stocking level, by treatment, was determined by calculating the percentage of cells that still had a tree present in 2007, regardless of past mortality.

Differences in levels of mortality attributed to *Armillaria* among the different root-removal treatments were evaluated by a chi-squared test at  $P \leq 0.05$ . If significant differences were detected with the overall chi-squared test in either the initially thinned, unthinned, or planted portion of the experiment, then the procedure of Goodman (1964) was used to determine those root-removal treatments, where levels of *Armillaria*-caused mortality differed significantly ( $P \leq 0.05$ ). Within each treatment, similar comparisons were made across the initial stand types (thinned, unthinned, and planted). Growth data were analyzed using a one-factor analysis of variance followed by Tukey's multiple comparison procedure ( $P \leq 0.05$ ).

To evaluate whether cells currently unstocked because of *Armillaria*-caused mortality in 2007 had experienced *Armillaria*-caused mortality before the 1992–1993 thinning to one tree per cell, cell counts were made across treatment and initial stand types (thinned, unthinned, and planted). If a cell was currently unstocked and had experienced *Armillaria*-caused mortality before the thinning, this cell was included in the “with” category. If a cell had not experienced mortality before the 1992–1993 thinning, the cell was included in the “without” category.

Spatial correlation among cells with mortality caused by *Armillaria* anytime during the study was tested across each treatment and replication using join-count statistics (Upton and Fingleton 1985). A contiguous spatial proximity chart was created in the form of a rectangular lattice, with 0 (no mortality in cell from *Armillaria*) and 1 (*Armillaria*-caused mortality in cell) for each treatment/replication. The spatial proximity was based on shared edges or corners of cells with *Armillaria*-caused mortality. Departure from a random distribution of mortality was detected by comparing the number of observed 0 and 1 joins between neighboring locations with the number expected for a random distribution. *Armillaria*-caused mortality was considered clumped in a treatment/replication, and the null hypothesis of mortality being random with no spatial correlation was rejected when  $P \leq 0.05$ .

## Results

### *Armillaria*-Caused Mortality

As with the 20-year results (see Table 2 in Roth et al. 2000), there was a general tendency for the more intense and thorough root-removal treatments to more effectively reduce the occurrence of *Armillaria* root disease (Table 1). However, even the most intensive treatment (treatment 1), which experienced significantly less disease than most other treatments, had 23% of the area expressing mortality over the 35 years. Currently, 85% of the area in this treatment remains stocked (Table 1). This level of disease occurrence compares

**Table 1. Percentage of cells with posttreatment mortality due to *Armillaria* through 2007 (35 years after root-removal treatment).**

Root removal treatment <sup>a</sup>	Thinned before 1992 <sup>b</sup>	Unthinned until 1992 <sup>b</sup>	Planted <sup>b</sup>	Pooled <sup>b</sup>	Stocking (%)
1–Push CR	25.5 AB	15.0, A	29.2, A	23.3, A	85
2–Push MR	21.6 A	27.7, AB	43.1, AB	29.8, AB	89
3–Push NR	28.0 AB	44.9, BC	57.4, B	40.3, BC	78
4–Push LS	24.7 AB	46.3, BC	44.4, AB	37.3, B	80
5–Fall NR	44.9 B	55.7, C	59.1, B	52.6, C	73
Pooled	28.9	37.9	46.6	36.1	81

<sup>a</sup> The treatments were as follows: 1–Push CR, trees pushed out, maximum removal of roots by machine, visible remaining roots picked out by hand; 2–Push MR, trees pushed out, maximum removal of roots by machine; 3–Push NR, trees pushed out, no further removal of roots; 4–Push LS, trees pushed out, large stumps left, otherwise maximum removal of roots by machine; 5–Fall NR, clear logged, sod scalped between stumps, stumps retained.

<sup>b</sup> Within columns, percentages followed by different letters (A–C) differ significantly according to Goodman (1964) confidence intervals ( $\alpha = 0.05$ ).

**Table 2. Percentage of cells unstocked in 2007 owing to mortality from *Armillaria*, with (w) and without (wo) a prior history of *Armillaria*.<sup>a</sup>**

Root removal treatment <sup>b</sup>	Thinned before 1992		Unthinned until 1992		Planted		Pooled	
	w	wo	w	wo	w	wo	w	wo
	..... (%) .....							
1–Push CR	7	93	50	50	58	42	32	68
2–Push MR	50	50	43	57	64	36	57	44
3–Push NR	38	63	71	29	38	62	44	56
4–Push LS	30	70	64	36	61	39	54	46
5–Fall NR	38	63	54	46	50	50	47	53
Pooled	28	73	57	43	53	47	47	53

<sup>a</sup> Prior history (w) means the cell experienced some mortality from *Armillaria* prior to 1992–1993, when all stocked cells were thinned to 1 tree per cell.

<sup>b</sup> Treatments are as defined in Table 1.

with nearly 53% of the area experiencing disease-caused mortality with no root removal, treatment 5 (73% of the area currently stocked), and just over 40% (78% currently stocked) with push over logging, treatment 3. Treatment 2 had an 89% stocking in 2007, and treatment 4 had 80% stocking.

Table 2 displays the cells that were unstocked in 2007 because of mortality from *Armillaria* with the history of previous mortality from *Armillaria* occurring (yes or no) prior to the general thinning in 1992–1993. Interestingly, in the originally thinned portion of the stand, there was a markedly greater tendency for cells experiencing mortality since 1993 (73%) to have low early mortality (28%), whereas the relationship was the opposite and not quite as strong in the unthinned and planted portions of the experiment.

In 10 of the 15 treatment/replication combinations (67%), including all three of the treatment 5 replications, as well as two of the three treatment 1, 3, and 4 replications, mortality caused by *Armillaria* was found to be clumped. That is, the cells expressing some mortality from root disease over the 35-year period were more likely to be adjacent to one another, rather than randomly distributed, in 10 of the 15 treatment blocks. We attempted to relate this clustering of mortality to disease levels present in the stand prior to installation of the treatments (see Figure 3 in Roth et al. 2000) but were unable to do so in a meaningful way. It is likely, however, that after 35 years the primary inoculum source is well abated and new infections are the result of secondary inoculum from the current crop of trees.

### Stand Growth

In general, the trees were growing well (mean annual increment of 0.29 m height and 0.53 cm dbh), and there were few significant differences in either height or diameter by root-removal treatment (Table 3). The growth differences present at age 21, prior to the “one tree per cell” thinning (see Table 5 in Roth et al. 2000), are now largely gone. Regardless of root disease treatment, the stand appears to have become more uniform in growth since thinning. We have no

**Table 3. Mean total height, dbh, and standard error, by treatment, of leave-trees 35 years after treatment.**

Root removal treatment <sup>a</sup>	Height (m) <sup>b</sup>	dbh (cm) <sup>b</sup>
1–Push CR	10.2 (0.3), A	18.7 (0.5), AB
2–Push MR	11.4 (0.2), B	19.8 (0.5), B
3–Push NR	10.2 (0.3), A	18.2 (0.5), AB
4–Push LS	9.8 (0.3), A	16.9 (0.6), A
5–Fall NR	10.1 (0.3), A	18.6 (0.6), AB
Pooled	10.3	18.4

<sup>a</sup> Treatments are as defined in Table 1.

<sup>b</sup> Within columns, means followed by a different letter are significantly different according to Tukey’s multiple comparison procedure ( $\alpha = 0.05$ ).

explanation for the significantly greater growth occurring in treatment 2 (Table 3), although, where planted, the Rogue River seed source performed exceptionally well in this treatment (Tables 4 and 5).

### Discussion

The general hypothesis that removal of inoculum can reduce levels of root disease is supported by these data in that overall the two most thorough levels of inoculum removal (treatments 1 and 2) expressed significantly less root disease-caused mortality after 35 years than the no-removal control (treatment 5). The only treatment that has promise as an operationally feasible, on-the-ground management action, treatment 3, also reduced levels of mortality, but not significantly (Table 1). Thus, from these data, it would be difficult to recommend this push-over logging treatment as an effective or economically viable management strategy for root disease control on these ponderosa pine sites.

The failure of push-over logging to provide effective control over time is somewhat surprising in that the efficiency of the technique in removing root biomass, as evaluated on other sites, was high: Omdal et al. (2001) concluded that no less than 83% of the estimated

**Table 4. 2007 mean height and standard error of surviving planted seedlings 35 years after treatment.**

Root removal treatment <sup>a</sup>	Seed source <sup>b</sup>		
	Deschutes	Local	Rogue River
	(m)		
1–Push CR	8.7 (0.7), A	9.2 (0.7), A	8.5 (0.6), A
2–Push MR	6.3 (0.6), A	6.3 (0.6), A	8.6 (0.6), B
3–Push NR	11.0 (0.9), A	8.8 (0.9), A	9.4 (0.7), A
4–Push LS	10.1 (0.9), A	9.3 (0.9), A	10.5 (0.8), A
5–Fall NR	7.1 (1.1), A	8.0 (1.1), A	9.5 (1.3), A

<sup>a</sup> Treatments are as defined in Table 1.

<sup>b</sup> Within a row, means with the same letter are not significantly different according to Tukey's multiple comparison test ( $P = 0.05$ ).

**Table 5. 2007 mean dbh and standard error of surviving planted seedlings 35 years after treatment.**

Root removal treatment <sup>a</sup>	Seed source <sup>b</sup>		
	Deschutes	Local	Rogue River
	(cm)		
1–Push CR	15.1 (1.4), A	17.5 (1.4), A	16.0 (1.2), A
2–Push MR	11.1 (1.2), A	11.7 (1.2), A	16.0 (1.2), B
3–Push NR	18.4 (1.5), A	16.0 (1.5), A	17.6 (1.3), A
4–Push LS	17.9 (1.4), A	16.3 (1.4), A	18.2 (1.4), A
5–Fall NR	12.5 (1.6), A	14.8 (1.6), A	18.9 (2.0), A

<sup>a</sup> Treatments are as defined in Table 1.

<sup>b</sup> Within a row, means with the same letter are not significantly different according to Tukey's multiple comparison test ( $P = 0.05$ ).

belowground biomass was removed. Furthermore, some 90% of broken roots remaining in the soil were less than 5 cm in diameter and should thus decay rapidly; 55% of the broken roots were less than 2.5 cm in diameter. Therefore, Omdal et al. (2001) concluded, “residual biomass remaining in the soil following stumping activities should not pose a significant disease threat other than possibly to seedlings planted directly in contact with infected root pieces,” p. 24.

To our knowledge, there are currently no ongoing, operational inoculum-reduction practices by stump removal for control of Armillaria root disease in forest stands in the United States and few, if any, for control of laminated root rot (caused by *Phellinus weirii*) (Thies and Westlind 2005). A site in New Mexico was prepared for an Armillaria root disease control trial by stumping (Schultz and Bennett 1994, Omdal et al. 2001), but its implementation after preparation was superseded by a pumice mining operation.

Kliejunas et al. (2005) report effective control of Annosus root disease (caused by *Heterobasidion* spp.) in a forest campground in California by stump removal and trenching, and Chastagner and Dart (2006) report successful control of this root disease by stump removal in a Christmas tree plantation; however, no operational activities for control of Armillaria or Annosus root disease are recorded for forest stands in the United States (Vasaitis et al. 2008). In contrast, stump removal has been widely applied in Europe and elsewhere for control of Armillaria and Annosus root diseases in forests with several examples of effectiveness, although economics of the practice may be questionable at times (Vasaitis et al. 2008).

We mentioned earlier a long-standing trial for control of Armillaria root disease in British Columbia (B.C.), Canada (Morrison et al. 1988, Morrison 1998). Promising results from this trial and others have led to development of operational guidelines for root disease control by root removal in the primarily mixed conifer forests of the interior of B.C. (Cleary et al. 2008). Interestingly, since 1991, more than Can.\$50,000,000 has been spent on root removal operations for root disease control (primarily for Armillaria root disease) in the southern interior B.C. (Michelle Cleary, B.C. Min-

istry of Natural Resource Operations, Jan. 28, 2011) (also see Westfall and Ebata 2010) which contrasts sharply with the fact that there has been no such application in any forest type in the adjacent United States. The B.C. root disease-control operations do not include any actions in the ponderosa pine type as Armillaria root disease is rarely damaging there, likely because of site dryness (Michelle Cleary, B.C. Ministry of Natural Resource Operations, Jan. 28, 2011).

In part because of the cost and limited effectiveness of operationally feasible control by root removal in the severely damaged forest we studied, current control practices are shifting species dominance away from ponderosa pine by planting more Armillaria root disease-resistant Douglas-fir and western larch (*Larix occidentalis*), (Blake Murphy, Washington Department of Natural Resources, Feb. 17, 2011). Even though the forest is in a transition zone from ponderosa pine to Douglas-fir, the forest composition is controlled by cold air accumulation (frost) and surface temperature extremes, conditions not particularly well suited to either of these alternative species. Interestingly, Douglas-fir is considered more resistant/tolerant of Armillaria root disease here than ponderosa pine, whereas the opposite seems true on many other sites (Shaw and Kile 1991, Frankel et al. 1998, Goheen and Willhite 2006).

Although not analyzed statistically, the planted portion of this experiment experienced greater levels of mortality in four of the five treatments, a finding consistent with other observations on root disease development in natural regeneration versus planted stock (Shaw and Kile 1991). The contrast in disease expression shown in Table 2 between the originally thinned and unthinned portions of the experiment after the universal thinning in 1992–1993 is difficult to explain. For example, the most effective treatment, treatment 1, in the originally thinned portion of the experiment expressed mortality almost exclusively in cells that had not experienced mortality prior to the universal thinning in 1992–1993. This result seems anomalous—particularly because the opportunity to detect disease from our bioassay approach was reduced by the general thinning in 1992–1993.



The general “clumping” of mortality as expressed by the likelihood of diseased cells to be adjacent rather than distant to one another is consistent with dynamics of root disease behavior. To determine whether clumping is being caused by a likely shift to tree-to-tree spread in the postthinning stand, rather than from residual inoculum, would require excavations like those done earlier (Reaves et al. 1993), when the trees were considerably smaller.

Recent detailed stem analyses of growth effects by *Armillaria* root disease on Douglas-fir (Cruikshank 2002) in B.C. have shown reductions in height and basal area of diseased trees. Our standing tree measurements, when analyzed by treatment rather than disease status of individual trees, show little difference across treatments. General tree performance meets expectations for this site (Barrett 1979), but our standing tree measurements do not show any real growth benefit by treatment. The exceptional performance by the Rogue River stock in the planted part of treatment 2 is an exception to this generality.

In conclusion, root disease remains the dominating disturbance factor affecting stand structure and threatening commercial forestry in this area. Although relatively long-term experimental results support the concept that inoculum removal will reduce root disease levels, the types of treatment that would have to be implemented to provide meaningful reductions in disease losses and gains in crop-tree stocking do not seem to warrant their cost. As such, the search for alternative means of control through a shift in tree-species composition continues.

## Endnotes

- [1] A name change to *Armillaria solidipes* has been recently proposed for *A. ostoyae* (Burdall and Volk 2008).

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